REMARKS

Claim Amendments:

Various claims have been amended to clarify the claims by changing the phrase "which has dicamba-degrading oxygenase activity" to "which catalyzes the oxidation of dicamba to 3,6-dichlorsalicylic acid (DCSA)". Support for this amendment is found on page 7, lines 13-14 (and is already found in previously presented claims).

Declaration of Donald P. Weeks Under 37 CFR 1.132

Enclosed herewith is a new Declaration of Dr. Donald P. Weeks under 37 CFR 1.132, which provides additional experimental evidence that: (1) one of skill in the art can, using the guidance provided in the specification and the knowledge in the art at the time of the invention, make modifications to the oxygenase of the present invention without destroying enzymatic activity; and (2) other species and genera of dicamba-degrading bacteria express oxygenases that fall within the scope of the present claims. Specifically, the new data provided by Dr. Weeks shows that changes in amino acids are tolerated in positions that are predicted by the known structural features of the oxygenase to be less important or non-important to the activity of the protein. In addition, the Declaration provides a further alignment of the complete amino acid sequence of the oxygenase of the invention with previously known oxygenases of a similar type and illustrates how the knowledge in the art regarding oxygenase structure allows one of skill in the art to modify the enzyme as presently claimed. Finally, the Declaration provides the amino acid sequences for oxygenases from three additional species of dicamba-degrading bacteria and shows the structural similarities to the $oxygenase_{DIC}$ represented by SEQ ID NO:4.

Objection to the Specification and Rejection of Claims 1, 2, 4, 5, 7, 21, 24, 36, 39, 44, 47, 48, 50-52, 54-56 and 58-65 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has objected to the specification and rejected Claims 1, 2, 4, 5, 7, 21, 24, 36, 39, 44, 47, 48, 50-52, 54-56 and 58-65 under 35 U.S.C. § 112, first paragraph, on the basis of written description. The Examiner maintains the position that the specification does not adequately describe

variants of the exemplified sequence of the dicamba-degrading oxygenase, or dicamba-degrading oxygenases from other bacteria.

Applicants traverse the Examiner's rejection of Claims 1, 2, 4, 5, 7, 21, 24, 36, 39, 44, 47, 48, 50-52, 54-56 and 58-65 under 35 U.S.C. § 112, first paragraph.

First, the Examiner states that while the specification describes how one of skill in the art could isolate other dicamba-degrading oxygenases or modify the exemplified dicamba-degrading oxygenase, the specification allegedly does not adequately describe isolated DNA molecules that fall within the breadth of the claims.

Applicants again disagree with this argument. The Examiner acknowledges that the specification teaches one of skill in the art the techniques required to isolate other dicamba-degrading oxygenases or modify the exemplified dicamba-degrading oxygenases. However, as set forth previously, the specification also teaches one of skill in the art about significant structural features of the enzymes and provides a reference to several other known oxygenases that have similar subunit structures, and which can be referenced for guidance in the knowledge of what structural features are correlated with the biological activity of the oxygenase of the present invention (see prior reference to the specification at page 40, line 27 to page 41, line 1; page 47, lines 12-15; page 54, lines 3-6; page 48, lines 1-18; Example 1). The Declaration of Donald P. Weeks filed on December 13, 2002 (i.e., the December 13 Weeks Declaration) provides additional evidence that by comparison to oxygenase structures known in the art, significant information regarding the structure of the claimed oxygenase can be determined. The new Weeks Declaration enclosed herewith provides an even more detailed illustration and discussion of the structure of the claimed oxygenase as compared to known oxygenases.

Second, the Examiner refers to Applicants' prior argument that the specification and the state of the art at the time of the invention provides guidance regarding the conserved structural features that would allow one of skill in the art to recognize what changes could be made to a given oxygenase without destroying the biological activity of the oxygenase. The Examiner contends that this argument was not persuasive because the "features" described in the instant specification are common to other P450 oxygenases and are not special technical features of the dicamba-degrading oxygenases.

Initially, it is noted that the oxygenase of the present invention is not in the family of cytochrome P450 oxygenases that use a heme group to transfer electrons. Rather, the oxygenase described in the present invention is a member of the Rieske non-heme iron-binding oxygenases that transfer electrons through iron-sulfur clusters and free iron atoms (see specification, Examples). In any event, Applicants traverse the Examiner's argument and submit that the Examiner is incorrect in dismissing as apparently irrelevant the structural features that are shared among all oxygenases of a given type. It is submitted that the Examiner can not dismiss what is generally known in the art about oxygenase structural characteristics that relate to the function of the enzyme. The issue at hand is whether one of skill in the art, given the specification and the knowledge in the art at the time of the invention, is equipped with sufficient guidance to be able to make changes in the oxygenase$_{DIC}$ structure and preserve the function of the enzyme. Structural features that relate to the function of the enzyme, **regardless** of whether or not they are shared with other oxygenases, are extremely relevant to this issue. It does not matter whether the mononuclear binding site, for example, is shared among several different oxygenases. This site is critical for the binding of exogenous mononuclear iron which is required for optimum oxygenase activity, as taught by Jiang et al., for example (e.g., see attachments to December 13 Declaration of Dr. Weeks under 37 CFR 1.132). Based on the alignment of multiple oxygenases, this site is highly conserved, particularly at selected positions, and therefore, one of skill in the art knows that modifications at these sites are not likely to be well tolerated if one wishes to maintain enzymatic activity. It does not matter that this is not a *unique* active site within the oxygenase$_{DIC}$ - it is a structure that is relevant to the functionality of a class of oxygenases *including the oxygenase$_{DIC}$*, regardless of the specific reaction catalyzed by the oxygenase, and it is information that one of skill in the art at the time of the invention can use to determine where in the protein modifications will be best tolerated. Therefore, this information is relevant to the issue of whether one knows what modifications to the claimed enzyme will affect biological activity. Applicants have already provided evidence that much was known about the structure of oxygenases that relate to the function of the enzyme type and have shown in the specification and through additional alignments provided in the December 13 Declaration and the new Declaration enclosed herewith that the oxygenase$_{DIC}$ shares such structural features so that one can predictably modify the structure within the scope of the claims and maintain enzyme activity.

Further on this point, and referring to the newly attached alignment of three previously known oxygenases with the oxygenase$_{DIC}$ of the present invention, it is well known in the art that simply by virtue of being an oxygenase, the oxygenase$_{DIC}$ of the present invention has the basic biological function of catalyzing the incorporation of molecular oxygen into a substrate to form a product. Therefore, one of skill in the art will expect a protein that falls within a class of proteins to share some structural features with the other proteins in the class that allow the protein to have this general function. For example, the oxygenases will all bind to a substrate, and even though the substrate differs among oxygenases and therefore the specific amino acids that interact with substrate should be different, the substrate binding region is expected to lie in a similar region of the protein among oxygenases. For example, in the case of Rieske non-heme iron-binding family of oxygenases (of which oxygenase$_{DIC}$ is a member), it is known that the portion of the substrate that is to be oxidized has to be in immediate proximity to the free iron atom that is a catalyst for the oxidation reaction, regardless of the identity of the substrate. Therefore, one of skill in the art would clearly avoid this site and the region in proximity to this site when making modifications. Moreover, within the broad class of oxygenases, there are subclasses with even more highly defined structure-to-function relationships. As discussed in the specification and further in the December 13 Weeks Declaration, the oxygenase$_{DIC}$ of the invention has an $(\alpha)_n$-type subunit arrangement similar to that found in several other well characterized oxygenases, and includes a Rieske iron-sulfur cluster and a mononuclear iron binding site. This is significant structural information that is known to be related to the oxygenase of the invention that can not be dismissed. Further computer-based comparisons of the complete amino acid sequence and predicted three-dimensional structure of oxygenase$_{DIC}$ with the known sequence and 3-D structure of naphthalene dioxygenase as described in the new Weeks Declaration, provides strong evidence that even those two Rieske non-heme iron-binding oxygenases with distinctly different substrates and primary amino acid sequences, both likely have highly similar tertiary structures both at the subunit and holoenzyme levels. Therefore, even though oxygenases may be distinct in terms of primary sequence and substrate binding, one can readily use structural information about the class of enzymes to make determinations about where to modify a specific oxygenase.

Applicants also refer to the attached new Weeks Declaration and submit that the data described therein provide even more evidence that the description provided in the specification, combined with the knowledge in the art at the time of the invention, is sufficient to allow one of skill in the art to selectively determine where modifications can be made in the protein that will avoid destroying the enzymatic activity of the protein. From the attached alignments, it is clear that up to 35% of the protein can easily be modified with the expectation that the protein will maintain biological activity (see discussion of Dr. Weeks in Declaration).

Moreover, the claims provide structure that distinguishes the variants of oxygenase$_{DIC}$ from other oxygenases by the limitations placed on the percent identity of the claimed oxygenase to the exemplified sequence (no other known oxygenase is even remotely close to the recited identity over the entire protein), and by the binding of the oxygenase to its specific substrate via the substrate binding region, or in the case of non-sequence based claims, by the recitation of specific structural/biochemical properties that are related to the function of the protein, and which have been used in the art to describe proteins for years.

For example, Claim 1 recites the molecular weight of the protein. Applicants consider the molecular weight of the enzyme to be a *structural* characteristic of the enzyme. Claim 1 also limits the protein from a dicamba-degrading bacterium, which places additional restrictions on the source and inherently on the structure of the oxygenase. Claim 1 also recites that the protein comprises an iron-sulfur cluster, which is a distinct structural feature of several oxygenases as discussed above, and which the alignments reveal is typically located in the same region of the full protein. This designation of oxygenase$_{DIC}$ as containing an iron-sulfur group also describes it as distinctly different from cytochrome P450 enzymes that possess heme groups for the transfer of electrons (instead of iron-sulfur groups) and yet can metabolize exactly the same types of substrates as Rieske non-heme iron-binding oxygenases. Claim 68 adds further structural features to the enzyme, a K_m for dicamba and a $V_{max.}$ The K_m of an enzyme is a kinetic (*i.e.*, rate) constant of the enzyme-substrate complex under conditions of the steady state. The structure of the enzyme is a significant factor in determining how the enzyme associates and dissociates with its substrate under various conditions and therefore, K_m is another characteristic of the oxygenase of the present invention which represents a known correlation between the structure and the function of an enzyme. The V_{max} is the maximum

initial velocity (i.e., the initial conversion rate of substrate to product by the enzyme). The structure of the enzyme significantly influences how it functions biochemically and therefore, V_{max} is a characteristic of the oxygenase which represents a known correlation between the structure and the function of an enzyme. Finally, the enzyme is described as catalyzing a specific enzymatic reaction, which is the functional characteristic related to the above-mentioned structural features.

Third, the Examiner contends that the specification describes only one isolated molecule encoding a dicamba-degrading oxygenase from a single organism and that this does not describe the genus of dicamba-degrading oxygenases. The Examiner refers to *University of California V. Eli Lilly and Co.* 43 USPQ2d 1398 (Fed. Cir. 1997) in support of this argument. The Examiner also contends that the argument that the specification describes the source of the protein, and the structural, biochemical and physical properties of the dicamba-degrading oxygenase is not persuasive because of the above-referenced case of *University of California V. Eli Lilly and Co.*

In response to these positions, Applicants submit that the case referenced by the Examiner has some significant differences from the present application. In the cited case, at issue was whether a description of the amino acid sequence for human insulin and a cDNA sequence encoding rat insulin was sufficient to support a claim to cDNA encoding vertebrate insulin and mammalian insulin. The claims contained no defining structural features of the claimed cDNA other than provision of a rat cDNA sequence and the claims placed no structural limits or boundaries on the cDNA other than the source of the DNA. The specification provided the cDNA encoding rat proinsulin and the amino acid sequence for human insulin.

In contrast, the oxygenase claimed in the present application is described in terms of a particular sequence or specific biochemical/physical properties; boundaries are set in the claims on what sequences are encompassed by the claims (e.g., via percent identity combined with a specific function or specific biochemical properties) and importantly, the specification provides a description and evidence of structural features that are associated with the activity of the enzyme and provides numerous examples of other oxygenases of similar type which can readily serve to inform the skilled artisan of structural conservation that is related to function. The specification also references other sources of dicamba-degrading bacteria from which similar oxygenases can be isolated. In the *University of California V. Eli Lilly and Co., ibid.*, the court concludes that it "may not be necessary

to enumerate a plurality of species if a genus is sufficiently identified in an application by 'other appropriate language'." Applicants submit that, for the reasons discussed in prior responses and herein, the specification provides sufficient guidance to identify oxygenases that fall within the scope of the claims to obviate the need to enumerate a plurality of specific additional species.

Furthermore, the structural, biochemical and physical properties of the dicamba-degrading oxygenase provided by the specification are specific characteristics to describe what is not believed to be a widely varying genus of enzymes. The claims are not simply directed to a cDNA encoding a dicamba-degrading oxygenase from any prokaryotic organism, or even from any bacterium, but rather to DNA encoding particular dicamba-degrading oxygenases from a particular source that has the specific structural and/or biochemical characteristics recited in the claims. As discussed above, the written description requirements do not demand that Applicants actually provide multiple species if the characteristics of the claimed molecules are identified in the application. The Examiner has not provided evidence as to why the genus of sequences encoding dicamba-degrading oxygenases from dicamba-degrading bacteria that have the recited characteristics would be expected to widely vary.

Fourth, the Examiner refers to the Weeks Declaration and the evidence that DNA from three different strains of bacteria contained DNA that was estimated to be greater than 90% identical based on the hybridization conditions used. The Examiner asserts that this data only provides asserted evidence of a possible function and sequence similarity, but not conclusive evidence of a dicamba-degrading oxygenase in other bacterial strains.

Applicants traverse this rejection. The data provided, including the rebuttal of the Examiner's position in the last response, does not provide evidence of a *possible* sequence similarity between the exemplified oxygenase and oxygenase sequences in other bacterial species, it provides definite evidence of a sequence in these bacteria that is highly homologous to the exemplified sequence. The fact that the use of hybridization conditions allow only for an *estimate* of the percent identity between sequences, simply means that the sequences are within a particular range of identity (i.e., 90-100% identical), which is nonetheless definite. However, this does not imply that there is some doubt that the sequences are highly structurally related. Hybridization has been used for years to identify and isolate highly related sequences. Moreover, because these bacteria are *dicamba-*

degrading bacteria (i.e., they have already been demonstrated to be capable of degrading dicamba), there is a strong correlation between the identified sequences and the inferred function of the protein encoded thereby given the functional data for the probing sequence supplied by the present specification. Applicants submit that the data provide strong evidence that the DNA identified in the other bacterial strains does encode a dicamba-degrading oxygenase within the scope of the invention, because to conclude otherwise given the data is simply not statistically or scientifically sound in view of the data. Applicants submit that this is yet another piece of strong evidence that supports Applicants' position, and that the Examiner appears to be viewing each data point in a vacuum instead of viewing the evidence as a whole.

However, to demonstrate that Applicants' prior hybridization data was not merely asserted evidence of a possible function and sequence similarity, the oxygenases from the three bacterial species described in the hybridization experiments have now been cloned and largely sequenced, and the data is provided in the Declaration of Dr. Weeks. These data show that, as predicted by the hybridization data, these oxygenases are between 90.1% and 99% identical at the amino acid level (and 98.1% to 99.9% identical at the nucleic acid level) to the corresponding portion of the $oxygenase_{DIC}$ represented in the present application by SEQ ID NO:4 (which is positions 15-324 of SEQ ID NO:4 for two of the enzymes or 15-326 of SEQ ID NO:4 for the other enzyme). Therefore, as predicted, other species and genera of dicamba-degrading bacteria express dicamba-degrading oxygenases that fall within the scope of the claims can be readily identified using the exemplified oxygenase.

In view of the foregoing remarks, Applicants respectfully request that the Examiner withdraw the rejection of Claims 1, 2, 4, 5, 7, 21, 24, 36, 39, 44, 47, 48, 50-52, 54-56 and 58-65 under 35 U.S.C. § 112, first paragraph.

Objection to the Specification and Rejection of Claims 1, 2, 4, 5, 7, 21, 24, 36, 39, 44, 47, 48, 50-52, 54, 55 and 58-65 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has objected to the specification and rejected Claims 1, 2, 4, 5, 7, 21, 24, 36, 39, 44, 47, 48, 50-52, 54, 55 and 58-65 under 35 U.S.C. § 112, first paragraph, on the basis of enablement.

First, the Examiner refers to Applicants' prior argument that the Examiner's position based on the teachings of Siminszky are not relevant, and submits that the argument is not persuasive because Claims 2 and 5, as an example, do not specify a specific activity of the encoded dicamba-degrading oxygenase. The Examiner again argues that Siminszky teaches that even though a P450 oxygenase reacts with a compound, there can be no expectation of a useful function.

In response to this portion of the rejection, it is initially noted that Claims 2, 5, and other claims referenced by the Examiner did previously recite a specific activity (i.e., dicamba-degrading oxygenase activity), which is believed to be sufficiently specific to distinguish this activity from other the activity of other oxygenases, for example. However, to expedite prosecution, Applicants have amended all of the relevant claims to more particularly recite the activity of "catalyzes the oxidation of dicamba to 3,6-dichlorsalicylic acid (DCSA)". With regard to the reference of Siminszky et al., Applicants again respectfully submit that while the Examiner's point is acknowledged, it is not directly relevant to the issue of whether an enzyme that degrades dicamba will predictably have utility in conferring dicamba resistance onto a host transformed with the enzyme. More specifically, in Siminszky et al., a P450 monoxygenase which was capable of catalyzing the metabolism of four different phenylurea herbicides (i.e. 4 different substrates) was only able to confer resistance in transgenic plants to two of the four herbicides. This is explained in Siminszky et al. as being related to differences in the relative toxicities of the four different substrates and the properties of the resulting metabolites from each substrate. Therefore, in Siminsky et al., the variation in the ability of the enzyme to confer tolerance on transgenic plants was not a variant property of the enzyme *per se*, but rather of the substrate (herbicide) used. In the present invention, there is only one substrate, dicamba, and the present inventors have clearly demonstrated that an oxygenase that metabolizes dicamba (i.e., by catalyzing the oxidation of dicamba to 3,6-dichlorsalicylic acid (DCSA)) confers dicamba resistance on a host transformed with the oxygenase. In the present invention scenario, the substrate (dicamba) is not a variable, and accordingly, the dicamba can be considered to be analogous to one of the two herbicides in Siminsky et al. against which tolerance was conferred on the transgenic plant. There is absolutely no reason provided by Siminsky et al. or in the argument of the Examiner as to why an oxygenase having the same biological activity (e.g., a functional variant of the dicamba-degrading oxygenase of the present

invention) would not be expected to also confer tolerance to a transgenic plant expressing the enzyme. It is clearly predictable from the data of record and the knowledge in the art that if the oxygenase catalyzes the degradation of dicamba, then dicamba-resistance is predicted and reasonably expected of the transformed host.

Finally, as discussed in the enclosed Declaration of Donald P. Weeks under 37 CFR 1.132 (paragraph 3; Discussion), the present inventors have now shown that transgenic plants expressing even low levels of oxygenase$_{DIC}$ activity are resistant to treatment with moderate to high levels of dicamba. From this observation, it is fully expected that even transformed plants expressing oxygenase variants having reduced enzymatic activity (instead of oxygenases with high specific activities), nonetheless, will confer dicamba-resistance on the host.

Second, the Examiner refers to Applicants' argument that because they have demonstrated that an oxygenase that degrades dicamba confers resistance on a host transformed with the oxygenase, there is no need to screen every dicamba-degrading oxygenase to see if it confers similar resistance to a host. The Examiner again refers to the alleged teachings of Siminszky and asserts that just because a P450 oxygenase can react with a compound does not inherently teach a useful function.

Applicants refer to the discussion of Siminszky above, and submit that, contrary to the Examiner's contention, a demonstration that an oxygenase that catalyzes the oxidation of dicamba to 3,6-dichlorsalicylic acid (DCSA) is capable of metabolizing dicamba and conferring dicamba-resistance onto a host transformed with the oxygenase, is sufficient to reasonably predict that enzymes having the same biological activity (i.e., catalyzing the oxidation of dicamba to 3,6-dichlorsalicylic acid (DCSA)) *against the same substrate* will also be able confer tolerance to that same substrate onto a host transformed with the enzyme). In addition, to respond to the Examiner's implication that there is no other useful function for the oxygenase of the invention, Applicants submit that the ability to confer dicamba-resistance onto a plant is not the only utility for a dicamba-degrading enzyme of the present invention. As discussed in the specification, one can transform microorganisms with the enzyme, to produce microorganisms that are capable of degrading dicamba. Such microorganisms can be used, for example, to degrade dicamba present in a material, such as soil, water, or waste products of a dicamba manufacturing facility (see page 24, lines 4-19). In

addition, the recombinant enzymes can also be purified from the recombinant microorganism and used directly to degrade dicamba present in such sources (see page 24, lines 4-19) or in a dicamba-detection assay of a sample (see page 25, lines 1-8). Neither of these uses necessarily require that the enzyme confer dicamba resistance onto an organism - instead, these uses only require that the enzyme have catalytic activity for the degradation of dicamba. Even so, Applicants have demonstrated that the oxygenase$_{DIC}$ of the present invention is capable of metabolizing dicamba and that microorganisms transformed with the enzyme are capable of metabolizing dicamba (e.g., see Examples and new Declaration of Dr. Weeks under 37 CFR 1.132). Applicants have also provided further evidence of the existence of oxygenases from other dicamba-degrading bacteria that fall within the scope of the claims (see again Declaration of Dr. Weeks). Variants of this enzyme having the same catalytic activity are predictably expected to have the same uses. Given these and the prior arguments of record, it is submitted that the specification has provided sufficient guidance to those of skill in the art to be able to predictably make and use a dicamba-degrading oxygenase as claimed in the current claims.

In further support of Applicants' position, the new Declaration of Dr. Weeks under 37 CFR 1.132 is again referenced. As discussed above, the data in the Declaration show that, given the description provided in the above-identified application and given the knowledge of the structure of oxygenases in the art, one of skill in the art can readily modify the oxygenase$_{DIC}$ gene of the present invention to cause directed changes in the amino acid sequence of the oxygenase$_{DIC}$ enzyme without destroying the enzymatic activity of the protein *in vivo*. In addition, the new Declaration demonstrates that oxygenases that fall within the scope of the claims can be identified in other dicamba-degrading bacteria.

In view of the foregoing remarks, Applicants respectfully request that the Examiner withdraw the rejection of Claims 1, 2, 4, 5, 7, 21, 24, 36, 39, 44, 47, 48, 50-52, 54, 55 and 58-65 under 35 U.S.C. § 112, first paragraph.

Applicants have attempted to respond to all of the Examiner's concerns as raised in the March 10, 2003 Office Action, and submit that the claims are in a condition for allowance. In the event that

the Examiner has any additional questions or concerns regarding Applicants' position, in an effort to expedite prosecution, contact of the below-named agent at (303) 863-9700 is encouraged.

Respectfully submitted,

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